DETERMINING FACTORS AFFECTING DERMO DISEASE (*PERKINSUS MARINUS*) IN POPULATIONS OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) IN GALVESTON BAY, TEXAS

A Thesis

by

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ABSTRACT

The Gulf Coast has seen a dramatic decline in commercial oyster harvest in recent years. Lack of fresh-water inflow and elevated temperatures and salinities have been identified as factors contributing to increased Dermo disease of oysters, caused by the parasite *Perkinsus marinus*, which attacks their tissue and is responsible for individual oyster and oyster reef kills along the Gulf Coast. A disease has its largest detrimental effect on a host when environmental conditions support an abrupt increase in density of the pathogen population. Therefore, documenting the relationships between distribution and prevalence of Dermo disease in the eastern oyster *(Crassostrea virginica)* and environmental conditions would be beneficial to management of the eastern oyster in Galveston Bay.

My study consisted of four sites located in Galveston Bay, Texas, which were sampled 20 oysters every other month from November 2014 through September 2015. Specific objectives were to determine: (1) prevalence of Dermo in oysters, (2) spatial location of Dermo infected oysters, (3) concentrations of the parasite Dermo within infected oysters (Mackin Dermo Intensity Scale), and (4) the relationship of water quality parameters (i.e., fresh-water flow, salinity, water temperature, and water turbidity) to prevalence and parasite concentration of Dermo disease in oysters.

Initially (November 2014), Dermo was present in oysters at all reefs sampled, and Dermo prevalence was greatest at April Fool (0.55, intensity on Mackin Dermo Intensity Scale) and Confederate (0.85) reefs, but declined after heavy rainfall (July 2015, April Fool Reef: 0.21, Confederate Reef: 0.81). Linear regression analysis

indicated water variables such as temperature, salinity, turbidity and fresh water inflow explained different amounts of the variability in the Mackin Dermo Intensity Scale among sampled reefs. Fresh-water inflow from the Trinity River explained the most variability in Dermo intensity at April Fool (61.8%), Fishers (44.5%), and Frenchy's (46.9%) reefs. At Confederate Reef, salinity (20.6%) explained the most variability in Dermo intensity, and this reef was least affected by the Trinity River flow. I found that combinations of low fresh-water inflow, high salinity, and high temperatures accounted for majority the variance of Dermo in oysters located in Galveston Bay. However, this relationship was not necessarily a linear relationship with mortality, in that high fresh-water inflow also was related to oyster mortality at Fishers Reef.

DEDICATION

I would like to dedicate this thesis to my family and friends who have encouraged and supported me throughout my academic experience. Particularly, I would like to dedicate this thesis and all of my future academic endeavors to my amazing mother, Dr. Valeen H. Silvy. I hope I can live up to your legacy.

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INTRODUCTION

Life History Characteristics of Eastern Oysters

The eastern oyster (*Crassostrea viginica*) is the primary species of commercially harvested oyster along the Texas Coast (Hedgpeth 1953). This bivalve mollusk, in the family Ostreidae, has two thick, flattened shells joined by a hinge controlled by a powerful abductor muscle (Kay 1979). The two valves of an oyster are asymmetrical with one valve being thicker and more deeply cupped than the other (Yonge 1960, Giltsoff 1964). Eastern oysters settle on substrate with thickest valve cemented to the substrate and the other valve on top (Eastern Oyster Biological Review Team 2007). Shell shape is variable depending on the environment in which the oyster grows (Eastern Oyster Biological Review Team 2007). On hard substrates the umbone of the oyster (a knob like protuberance arising from the surface near the hinge of the oyster shell) is curved and points towards the posterior end of the shell and shells are thick (Easter Oyster Biological Review Team 2007). When grown in silty environments the umbone grows straight out from the hinge and generally shells are thin and more fragile as compared to oysters on hard substrates (Easter Oyster Biological Review Team 2007). The eastern oyster shell has a prominent purple-pigmented scar inside the shell, where the abductor muscle is attached near the posterior end (hinge); this differentiates the eastern oyster from similar species (Easter Oyster Biological Review Team 2007) such as the Pacific oyster (*Crassostrea gigas*).

Oysters live up to 20 years (Buroker 1983), and are protandric, sexually maturing first as males and then changing to females after the first spawn, but can alternate sexes

throughout their lives (Thompson et al. 1996). Studies of factors that determine sex in the eastern oyster are inconclusive and indicate a complex underlying process. Oysters can change sex annually in response to a combination of stress factors related to environment, nutrition, and/or physiology (Tranter 1958). Reproduction is by external fertilization and spawning can occur when temperatures warm above 20^{o}C, usually late June to November (Dame 1972). Fertilization occurs in open water as oysters expel gametes into the water surrounding their home reefs. The presence of sperm or eggs in the water column stimulates the release of gametes by other adult oysters (Kennedy 1982). A female oyster can produce from 15- to 114-million eggs during a single reproduction cycle, which can occur once a year or more often, depending on environmental factors (Buroker 1983). The fertilized egg develops into a free-swimming trochophore, which does not feed. Within 24–48 hours the trochophore develops into a free-swimming veliger larvae which has a thin transparent shell and feeds on phytoplankton (Wallace 2001). After two to three weeks the veliger larvae develops into a pediveliger with a distinct foot and eyespots and begins to explore appropriate substrate on which to settle (Wallace 2001). The oyster uses its foot to detect adequate substrate such as cultch (dead oyster shell) and river rock, on which to settle (Kennedy 1996); in the Gulf of Mexico this occurs between July and December (Buroker 1983). This small oyster, called a spat, settles on suitable substrate, attaches itself, and then can grow, reaching sexual maturity in four weeks (Wallace 2001). Substrate for attachment depends on local availability and can be cultch (oyster shell) or rock and gravel substrate. The growth rate of the eastern oyster depends on temperature

and food availability (Kennedy 1996). They attain harvest size (75–90 mm) in the Gulf of Mexico 18–24 months after attachment (Hofstetter 1977). In comparison, oysters in the northeast region of the United States may take up to five years to reach a harvestable size (76–90 mm; Shumway 1996). One valve, commonly the upper, of the oyster shell grows faster than the other (Carriker 1996). Oyster growth continues throughout the life cycle, but the rate declines with age (Carriker 1996). In the Gulf of Mexico, oysters may reach 25–30 years in age and up to 30 cm in length (Martin 1987).

Young oysters depend on healthy plankton communities for food during the veliger larvae stage and as spat. Adult oysters feed primarily by extracting phytoplankton and detritus from the water that they filter through their gills (Langdon and Newell 1996). However, phytoplankton is the only required food source for gametogenesis (Kennedy 1996). They have small laterofrontal cilli that retain 1–30 μ-sized particles. Oysters can filter up to 6.8 L of seawater per hour (RiisgErd 1988), although some studies suggest that oysters can filter up to 36 L per hour (Brusca and Brusca 1990). As sessile organisms, oysters rely on water currents to move food-laden water past the gills where phytoplankton is extracted. Water flow (velocity) directly effects optimal growth rate (Langdon and Newell 1996). Too much flow causes the food source to move out of the accessible area before it can be extracted from the water column, whereas low flow rates can decrease the amount and rate of delivery of food (Grizzle et al. 1992).

The eastern oyster can tolerate wide swings in temperature, salinity, turbidity, and dissolved oxygen (Kennedy 1996), but these factors have a large influence on oyster

growth. The minimum temperature required for growth in oyster larvae is 17.5^{o} C (Hofstetter 1977). Although, at temperatures above 35^{o} C, oysters show greatly reduced filtration rates, and therefore reduced feeding rates (Loosanoff 1958, Giltsoff 1928). Oysters in the Gulf of Mexico can grow all year long, but optimal temperatures range from 20 to 30^{o} C (Stanley and Sellars 1986). Oysters can tolerate salinities from 0 to 42 ppt, but the optimum range for maximum growth to reproductive size and for reproduction occurs between 15 and 28 ppt (Quast et al. 1988, Shumway 1996). Mature oysters can suffer high mortality when salinities fall below five ppt for extended periods of time. The exact length of this time period is unknown, but it has been suggested that at least a 48 hour period of salinities below five ppt can cause mortality (Quigg et al. 2010). Also, high water temperatures may exacerbate the negative effects of low salinity leading to massive reef die-offs (Shumway 1996).

The Importance of Eastern Oysters to Their Ecosystem

Oysters serve as a key structural component of estuaries and bays (Berquist et al. 2006), and they play a major role in the functioning of bay and estuary ecosystems (Dame 1972). Oyster beds provide habitat for many invertebrate and fish species and serve as physical filters that remove particles from the water as it flows over reefs, and thus, reduce water turbidity (Meyer and Townsend 2000, Berquist et al. 2006). Oyster reefs promote species diversity and community stability by enhancing habitat value and affecting water circulation and flow patterns that improve water quality and nutrient recycling (Easter Oyster Biological Review Team 2007). Due to their high filtration

rates, eastern oysters have been considered a bioremediation tool to reduce contaminants in marsh-estuarine systems (Breitburg et al. 2000).

Oysters provide vital resources to both invertebrate and vertebrate marine communities. Oyster reefs provide a valuable refuge for organisms at various trophic levels. Their function is similar to that of submerged vegetation in that they provide physical habitat used by many fish species where sea grasses are not abundant (Holt and Ingall 2000). Bahr and Lanier (1981) documented more than 40 macrofaunal species or taxonomic groups that reside on oyster reefs, but the total number of species may exceed 300 (Wells 1961). Crabs, shrimp, isopods, amphipods, polychaetes, gastropods, sessile invertebrates, and sponges are found in oyster reef habitat (Eastern Oyster Biological Review Team 2007).

Suspension feeding by oysters provides not only bottom-up support for consumers at higher trophic levels by converting detritus to animal biomass, but also to lower-level, primary producers as oysters mineralize carbon and release nutrients into more-available forms (GSMFC 2004).

Reef configurations vary in size and shape, ranging from fully submerged to intertidal. An oyster reef consists of a colony of living, market and below market sized oysters, which may or may not be open to commercial and public harvest (Shipley and Kiesling 1994). Oyster reefs in the Gulf of Mexico can extend multiple kilometers, or consist of small isolated remote mounds (Robinson 2015). Oysters located in soft sediment environments that have a population density considered to form a reef, can aid in controlling underwater and shoreline erosion, by serving as breakwaters (Piazza et al.

2005). Oyster reefs can sub-divide bays and change water circulation patterns (Diener 1975). In turn through feedback mechanisms, these reefs are altered by changing patterns in bay water circulation. In the northern most range of oysters, freezing temperatures during winter months limit growth of reefs occur only in subtidal environments. In the mid-Atlantic, reefs extend higher into the water column where plankton is most dense (Lenihan and Peterson 1998). However, reefs in the mid-Atlantic region are notably smaller and less dense now than historical references would suggest, likely due to overharvest (Rothschild et al. 1994). In ecosystems where oysters are overharvested, their growth and survival rates may be more susceptible to the influence of pathogens and temperature than in systems where commercial harvest rates are low (Eastern Oyster Biological Review Team 2007). Although many eastern oyster reefs found in the southeast Atlantic coast of the United States and in the Gulf of Mexico are completely subtidal there are also many that extend into the intertidal zone. In the Gulf of Mexico, oysters are found in water from 0 to 4-m deep (MacKenzie and Wakida-Kusunoki 1997). In the subtidal, high recruitment reefs, population growth is limited primarily by food availability and reproduction. On these high-density reefs, predation and disease are stronger negative impacts on survival than they would be for low density reefs.

Oyster Populations in the United States

The eastern oyster is found along the eastern coast of North America from the Gulf of Saint Lawrence to the Caribbean, and is the dominant species of oyster in the Gulf of Mexico (Kennedy 1996). It has been introduced into Hawaii, the west coast of

North America, and numerous other locations worldwide (Maryland Sea Grant 2015). Historical information on abundance and reef densities within estuaries are often vague (Ingersoll 1881). Historic abundance is often hard to pinpoint because of a lack of reliable quantitative survey data in many regions. Current research often contests earlier published estimates of abundances and local reef placement (Eastern Oyster Biological Review Team 2007). The reason behind this is that earlier surveys focus more on harvestable reefs (Eastern Oyster Biological Review Team 2007).

Abundance of the eastern oyster has declined in many estuaries (zu Ermgassen 2012). For example, some populations are now considered "ecologically extinct", and no longer serve as a keystone species where they formerly were abundant (Eastern Oyster Biological Review Team 2007). In many places, isolated oysters can be found resting upon a sediment layer that once was a dense oyster reef. Decline of oyster abundance has most often been observed in urbanized areas that are associated with long periods of exploitation, such as New England (Eastern Oyster Biological Review Team 2007). In the Gulf of Mexico however, local population decline have usually been attributed to varying environmental conditions (Eastern Oyster Biological Review Team 2007).

Gulf Coast Oysters

Total harvest of the eastern oyster in the Gulf of Mexico comprises 70% of the United States total oyster harvest, and yield approximately 90,718,466 kg (22 million pounds) of meat per year (Puglisi 2008). Most of the Texas production of oysters is on private leases, whereas in other Gulf States production comes mostly from public reefs

(Eastern Oyster Biological Review Team 2007). Florida and Alabama only allow tongs for harvesting oysters from public reefs, while Mississippi, Louisiana, and Texas allow harvest using dredges. Florida, Louisiana, and Texas are the only states that market oysters year round. Historically along the Gulf Coast, oyster landings increased during the 1960s and 1970s, peaked in the early 1980s, declined into the early 1990s, and have been stable with slight declines since then (Eastern Oyster Biological Review Team 2007). Loss of suitable oyster habitat and water quality have been the leading causes of recent decline in oyster harvest, along with other detrimental activities such as shrimp trawling, oil and gas structures, channelization and dredging, and fresh-water diversions impacting oyster growth and sustainability (Dugas et al. 1997).

Throughout the Gulf Coast, oyster health and growth is monitored by state agencies and voluntarily reported on a disease monitoring website (oystersentinel.org). The Oyster sentinel website contains records from oyster sampling at various locations along the Gulf Coast that have been tested for the presence of Dermo disease, a sometimes fatal oyster disease caused by a protozoan parasite (*Perkinsus marinus*) that infects the oyster's tissue and causes mass die offs and reef kills. The Texas Department of State Health Services also monitors oyster reefs for bacteria and fecal matter and establishes harvest regulations to assure reefs safe to harvest oysters for the purpose of human consumption (Texas Department of State Health Services 2015).

The Texas oyster industry is the only state that consists of both public reefs and private leases. These leases contain oysters that have been legally transplanted from waters where harvest is restricted. Lease harvest comprises 25% of Texas oyster harvest

annually. There are 930 ha of private oyster leases in Galveston Bay (Robinson 2015). This system of oyster leases is currently only used Galveston Bay (Eastern Oyster Biological Review Team 2007). Oyster density on Galveston Bay reefs are currently monitored by Texas Parks and Wildlife Department (TPWD) using a standard-size (76-mm mesh) oyster dredge which is pulled behind a boat for 60 seconds in a randomized pattern across all known reef locations (HARC 2010). Since the 1950's, Galveston Bay has produced 80% of the oyster harvest in Texas (Lester and Gonzales 2011). In 1976, Galveston Bay had 3,045 ha of surveyed oyster reefs (Quigg et al. 2010). In 1994, a survey documented 5,750 ha of oyster reef, excluding west Galveston Bay (Quigg et al. 2010).

Published Houston Advanced Research Center (HARC) maps (Fig. 1) of oyster reefs in Galveston Bay (1954 to 1958), document a steep decline in the number of oyster reefs. By 1980 even fewer oyster reefs remained in Galveston Bay. Reefs open to harvest in 2009 (Fig. 2) represent a 50% decrease from those open in the1954–1958.

Threats to the Eastern Oyster in Galveston Bay

The leading cause of oyster reef decline is a loss of suitable shell base habitat (Eastern Oyster Biological Review Team 2007). Previous overharvest and unwise management decisions on both public and leased oyster reefs play an integral role in reef decline. Cultch (or oyster shell and rock) is not replaced once it is removed from a reef, and thus greatly reduces the remaining area of hard bottom habitat available for spat to settle and attach itself. These abiotic and biotic factors have served to fragment remaining reef habitat (Eastern Oyster Biological Review Team 2007). The reduction in

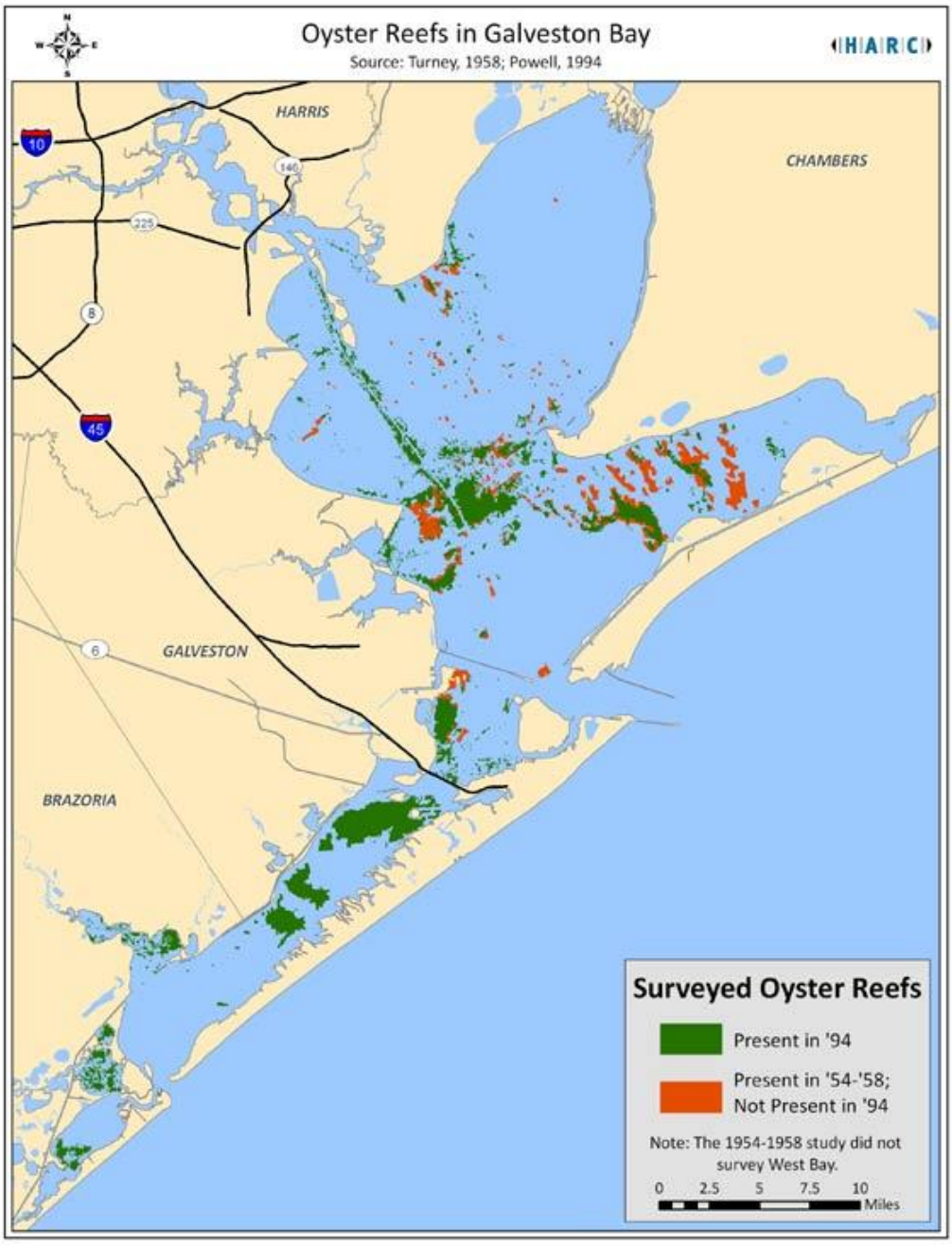

Figure 1. Map showing oyster reefs present in 1954–1958 and absent in 1994 (from HARC 2015).

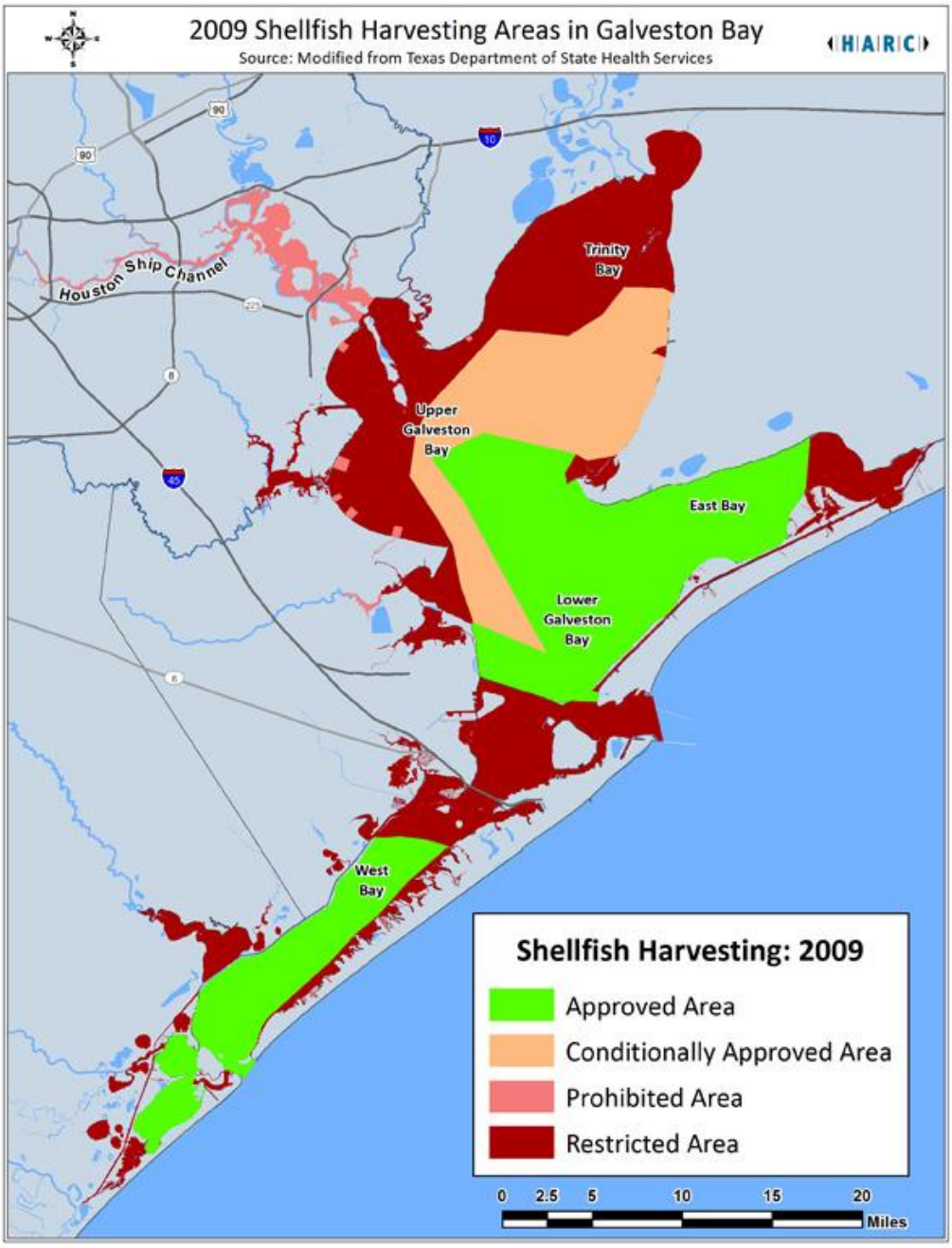

Figure 2. Oyster reefs open for harvest during the 2009 season (from HARC 2015).

oyster density reduces the ability of a reef to serve as a source of gametes and larvae to surrounding reefs. Aquaculture-based fisheries that occur in or near an oyster reefs, also compete with oysters for habitat and other resources (Burrell 1997), and contribute to an excess of waste products that may impair water quality and oyster survival (Serve et al. 1971). Another factor contributing to reef habitat decline is the increased pressure placed on harvestable reefs when others are closed due to contamination (Eastern Oyster Biological Review Team 2007).

A second threat to eastern oyster populations throughout their range is the decline in water quality, caused by both abiotic and biotic factors. Eutrophication, caused by inadequately controlled nutrient inputs creates an excess of phytoplankton biomass, which can lead to hypoxic or anoxic conditions. Eutrophication also can increase the incidence of toxic or harmful algal blooms that can cause reef die-offs (Giltsoff 1964). Dense mats of algae can cover and smother shallow oyster reefs (Giltsoff 1964). Improper waste disposal can generate large quantities of toxic chemicals that inhibit oyster growth (Giltsoff 1964). The sensitive larval stage of oysters is highly susceptible to the negative impacts associated with exposure to suspended sediments and associated turbidity (Davis and Hidu 1969). Suspended sediments can cover oyster reefs and decrease oyster abundance (Giltsoff 1964).

Natural variation in other environmental factors can also limit oyster population growth. Projected increases in water temperature associated with climate change could alter and limit the distribution of oysters, their predators, and diseases, especially at extreme temperatures (Eastern Oyster Biological Review Team 2007). Severe weather

events, such as drought or hurricanes can physically alter oyster beds or create noxious temperature and salinity conditions (Berrigan 1988, Dugas et al. 1997, Perret et al. 1999). Introduction of invasive species, such as the Asian gastropod mollusk (*Rapana venosa*), can cause competition for suitable habitats and food sources (Mann and Hardy 2003). These invasive species also can prey upon larval and adult oysters (Mann and Hardy 2003).

Oyster Diseases

Oyster disease is a reoccurring problem affecting the viability of oyster reefs of Galveston Bay. Two pathogens, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo disease) which are spore forming protozoan parasites have caused massive reef die-offs in populations of the eastern oyster in Galveston Bay (Heare 2008). Not much information is known about the life cycle of MSX, but the major life stage is a multinucleated plasmodium which infects the oyster tissue (Heare 2008). Dermo disease, previously identified as, *Dermocystidium marinum*, caused by *Perkinsus marinus* which has three life stages, which can cause infection in oysters (Andrew 1988 and Galstoff 1964) and eventually death. Oyster death is caused by Dermo spores which grow within oyster tissue and eventually lyses it (Heare 2008). Dermo disease is transmitted from an infected oyster to surrounding oysters when decomposing tissue from dead oysters releases spores into the water column (Audemard et al. 2004). Although a potentially fatal disease to oyster populations, Dermo is harmless to humans (Quigg et al. 2010).

The earliest known incidence of Dermo in oysters was reported at the 1893 Chicago World's Fair in oysters shipped from Louisiana. Tissues from some of these oysters, which had been stored and preserved by New Orleans' Cabildo Museum, where examined, and parasitic spores were found (Heare 2008). Dermo was later described by Mackin et al. (1962) based on examination of infected oysters from Gulf States (Heare 2008). Activity of Dermo increases at high salinities (>10 to 12 ppt; Heare 2008). This usually occurs due to reduced rainfall or freshwater discharge from coastal rivers that ultimately lead to an increase in salinity, which triggers a rise in Dermo disease prevalence and intensity, producing increased oyster mortality (Soniat et al. 2012). Temperature also is a key factor affecting the prevalence of the disease, because pathogen growth is halted below 20^{o} C (Hofmann et al. 2001) and magnified above 20^{o} C. A decrease in fresh-water inflow during warmer months can lead to combined increased salinity and temperature potentially leading to an increase in Dermo activity (Ewart and Ford 1993, Culbertson 2008).

Dr. Sammy Ray, of Texas A&M University–Galveston along with his former Ph.D. student, Dr. Thomas Soniat, studied the relationship between water temperature and salinity to the prevalence of Dermo in oysters from the Gulf Coast during the 1990s through the early 2000s. From 1998 until his death in 2011, Dr. Ray continued to monitor the prevalence of Dermo disease in oysters from Galveston Bay using the modified Ray's fluid thioglycollate method (RFTM; Mackin 1962, Mackin and Way 1952). This method involves the examination of oyster tissue that has been stained using Lugol's solution. The monitoring results are provided on oystersentinel.org, a web

based database which lists the results of pathogen monitoring using the eastern oyster to monitor the health of oyster reeds along the Gulf of Mexico. The web site provides historical temperature and salinity readings, as well as Dermo prevalence in market and under market sized oysters (Oystersentinel.org 2015).

OBJECTIVES

To better understand the effects and relationships between environmental variables and Dermo disease on the eastern oyster population in Galveston Bay, I collected oysters from 5 sites within Galveston Bay to determine the prevalence of Dermo disease bimonthly for a year long period from November 2014 to September 2015. Specific objectives of my study were to determine: (1) prevalence of Dermo disease in oysters, (2) spatial location of Dermo infected oysters, (3) concentrations of the Dermo within infected oysters, and (4) effects of water quality (i.e., fresh-water flow, salinity, water temperature, and water turbidity) on prevalence of Dermo disease in oysters in Galveston Bay.

STUDY AREA

All reefs that were sampled were located are north of Galveston Island and Bolivar Peninsula (Fig. 3). These reefs were chosen because each of them represents a different section of the estuary where oysters are normally produced including the northwest (April Fool Reef), southwest (Confederate Reef), northeast (Fishers Reef), and southeast (Frenchy's Reef, alternate: Hannah's Reef) in Galveston Bay. I collected oysters at four study sites (April Fool Reef [29.476666, -94.914322], Fishers Reef [29.658300, -94.838800], Frenchy's Reef [29.527800, -94.606900], replaced later with Hannah's Reef [29.478459, -94.726181], and Confederate Reef [29.263208, -94.917583]). Researchers and technicians from Dr. George Guillen's lab (Environmental Institute of Houston, University of Houston at Clear Lake) provided boats and crew to access sample sites. All samples were collected under Dr. Guillen's permit (SPR-0504-383) from TPWD.

April Fool Reef

Located south of the city of San Leon, Texas, April Fool Reef is approximately a five minute boat ride from the city. It was accessed from the boat ramp at the Topwater Grill in San Leon. It was chosen due to its proximity to the Houston Ship Channel and the possible effects of boat traffic and turbidity to the reef. April Fool Reef was sampled six times bimonthly from November 2014 to September 2015. It is characterized as an "alongshore reef" (Powell et al. 1995). It was perhaps historically a part of the chain of

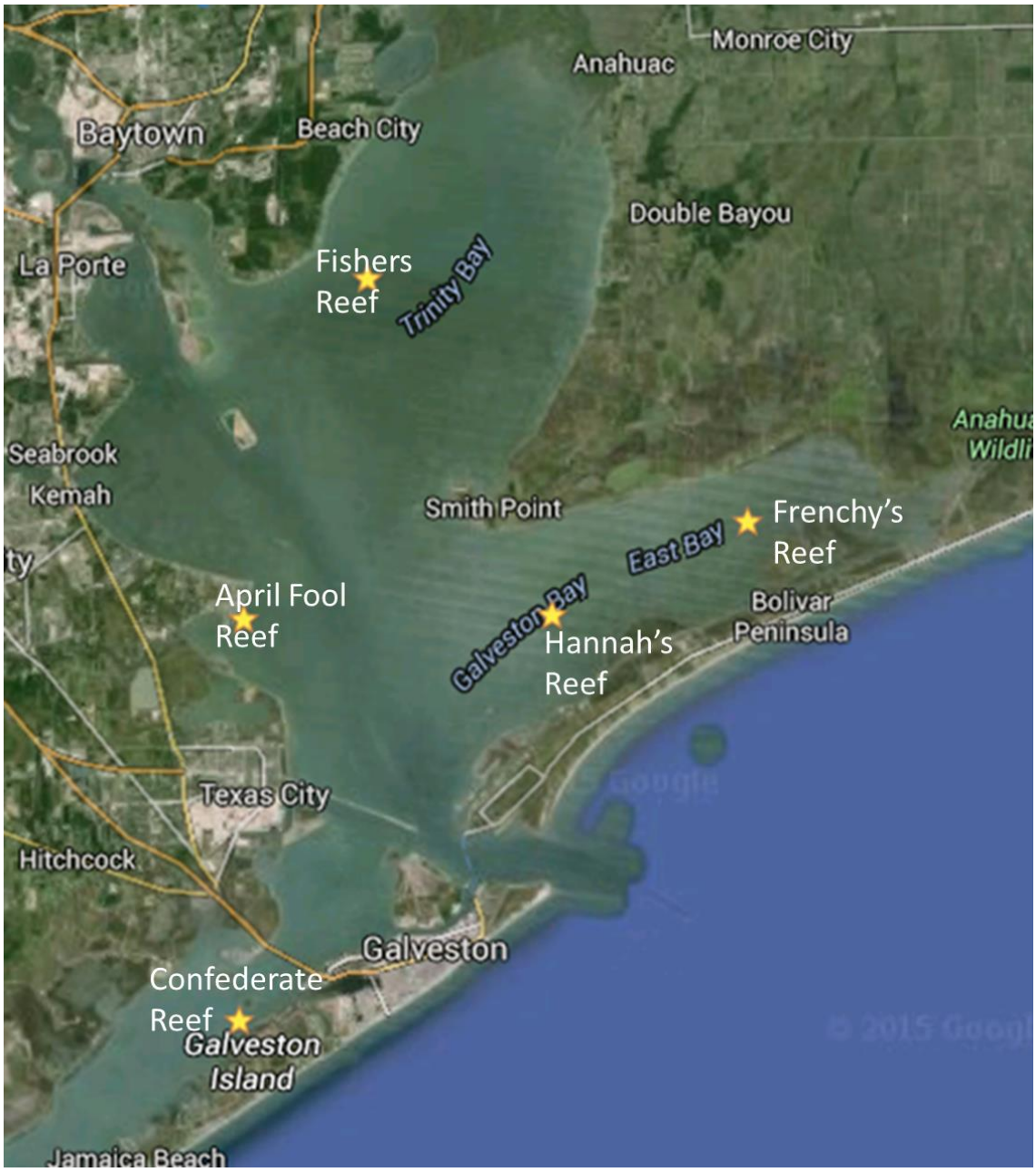

Figure 3. Locations of April Fool Reef, Fishers Reef, Frenchy's Reef, replaced later with Hannah's Reef, and Confederate Reef where oysters were collected (created from Google Earth 2015).

reefs known as Redfish Reef, which culminates in Redfish Island and became divided into smaller reefs as a result of dredging (Powell et. al. 1995). Historical Dermo data, including temperature, salinity, and Dermo prevalence are available for this reef from 1998 through 2011. (http://www.oystersentinel.org). Prior to this study, oysters were collected and processed by Dr. Sammy Ray, (Professor, Texas A&M University–Galveston) from this site. Historical salinities have ranged from 2.0 ppt (June 2001) to 32.0 ppt (October 1999). Water temperatures recorded at the reef have ranged from 9.8° C (January 2003) to 32.8° C (August 1999). This reef has a history of Dermo infection in market sized oysters which peaked during November 1999 with a prevalence of 2.87(http://www.oystersentinel.org).

Fishers Reef

Fishers Reef is closest to the mouth of the Trinity River and the Houston Ship Channel and selected because of its proximity to a source of fresh-water inflow. It was accessed within 15 minutes by boat from Thompson's Boat Ramp and Marina in Baytown, Texas and sampled six times bimonthly from November 2014 to September 2015. Fishers Reef is characterized as a transverse ridge reef (Culbertson 2008). Dermo data, including temperature, salinity, and Dermo prevalence are available on this reef from 1998 through 2011. (http://www.oystersentinel.org). Historical salinities have ranged from 0.2 ppt (July 2007) to 32.7 ppt (September 2011), and water temperatures have ranged from 7.6° C (January 2010) to 32.8° C (August 2003; oystersentinel.org). It has consistently shown Dermo prevalence levels under 1.0 (Mackin Dermo Intensity

Scale [hereafter Mackin Scale]; Mackin 1962) since 1998, with the only exception in September 2011 when it was 3.53 (oystersentinel.org).

Confederate Reef

Confederate Reef is located in West Galveston Bay and was accessed by a public boat ramp at the end of 8-mile Road in Galveston, Texas. It was selected because it is a tidal reef, submerged at high tide and exposed at low tide. Confederate Reef was sampled six times bimonthly from November 2014 to September 2015. Dermo data, including temperature, salinity, and Dermo prevalence are available on this reef from 1998 through 2011. (http://www.oystersentinel.org). Historical salinities have ranged from 8.7 ppt (June 2015) to 42.0 ppt (August 2009). Temperatures have ranged from 6.0° C (January 2010) to 36.0° C (August 2006). Confederate Reef has shown high levels of Dermo prevalence consistently from 2008 until present with levels of Dermo prevalence above 0.33 (Mackin Scale) until June of this year. It reached its peak Dermo prevalence of 3.03 (Mackin Scale) in August 2010.

Frenchy's Reef

Frenchy's Reef has been a commercially harvested oyster reef since at least 1966 (Hofsetter 1966). It was chosen because it is a public reef, and susceptible to the pressures of commercial fishing, unlike the other reefs sampled. It is located north of the Bolivar Peninsula (oystersentinel.org). It was accessed from the Stingaree Restaurant Boat Ramp and sampled only four times bimonthly from November 2014 to May 2015, at which time it was replaced with an alternate reef (Hannah's Reef) after dredging

efforts at Frenchy's Reef yielded no live oysters. Frenchy's Reef was approximately a 15 minute boat ride from the boat ramp. It was part of a $3.8 million reef restoration effort in 2011, in which 53,519 $m^3$of cultch (oyster shell and river rock) were spread over 72 ha of public reef (Rohrer 2011). Water temperature, salinity and Dermo prevalence data are available on this reef from 1998 through 2011 (http://www.oystersentinel.org). Historical salinities have ranged from 2.1 ppt (October 2002) to 28.0 ppt (March 2000). Water temperatures have ranged from 8.1° C (January 2003) to 31.3 °C (August 2003). Dermo prevalence levels have never reached above 1.96 (Mackin Scale) except in June 2011, when it was 2.06 (oystersentinel.org).

Hannah's Reef

Hannah's Reef was selected as the alternative site to Frenchy's Reef (commercially harvestable reef, see above) and because of its close proximity to Frenchy's Reef in Galveston Bay. Hannah's Reef was chosen as an alternate because it is closed to commercial harvest and oysters were presumed to be more readily collected. It was sampled twice, once in each June 2015 and September 2015. Water temperature, salinity, and Dermo prevalence data are available on this reef from 1998 through my collections from November 2014 to September 2015 (oystersentinel.org). Historical salinities have ranged from 4.0 ppt (November 2002) to 30.0 ppt (March 2000). Water temperatures have ranged from 8.1° C (January 2010) to 31.1° C (August 2010). Dermo prevalence reached its peak at Hannah's Reef in September 2010 with a prevalence level of 2.87 ((Mackin Scale; oystersentinel.org).

FIELD METHODS

Oyster Collection

Boats used included a 6.7 m Twin Vee with a 2012 (130 hp) Evinrude E-tec motor, and a 6.7 m JH Performance with a 2009 (150 hp) Yamaha (4 Stroke) motor. I sampled each site every other month starting in November 2014 and ending in September 2015.

A 30 x 30-mm mesh size oyster dredge (provided by Dr. Thomas Soniat, University of New Orleans; Fig. 4) was pulled behind a boat for three to ten minutes in slow circles and repeated three to eight times as necessary to collect a total of 20 market-sized and smaller oysters. If a reef was accessible by wading than 20 oysters were collected by hand. Oysters were placed on ice in a cooler for up to 24 hours until the samples were processed.

Location of sample sites was determined using a boat-mounted Hummingbird 1158C model GPS. Salinity (0.1 ppt) and water temperature (0.1° C) were measured using an YSI pro plus meter one foot below the surface. At each site, water quality data were collected during each oyster collection. Turbidity was recorded using a Secchi tube (0.1 mm).

Oyster Sample Processing

Once collected, oysters were taken to Dr. George Guillen's lab in Clear Lake, Texas for processing. Oysters were numbered from 1–20 for each site separately. Each numbered oyster shell was measured (Fig. 5) from hinge to beak with calipers (0.1 mm). Data recoded for each oyster included the following: date of collection, date of processing, and bill

Figure 4. Oyster dredge used to collect oysters from oyster reefs in Galveston Bay.

condition of each oyster (as either sharp [indicates new growth, sharp to the touch at the edge of the beak of the oyster shell] or dull [indicates no new growth, smooth to the touch]; Fig. 6). Each oyster was shucked using an oyster knife and gloves. The oyster meat was left in the cupped half shell and meat condition was recorded (as either shrunken [small, shrunken, dehydrated appearance] or plump [round, lush, creamy]; Fig. 7). These meat conditions were based on descriptions from Ray's 1966 methods.

LAB METHODS

To detect Dermo in the oysters, I first had to prepare a Thioglycollate culture medium, antibiotics, and a Lugol's working solution. This was done in Dr. George Guillen's lab in Clear Lake, Texas.

Preparation of Thioglycollate (Thio) Medium

In 1952, Dr. Sammy Ray (Texas A&M University at Galveston, Texas) developed the Thioglycollate culture method for detecting *Dermocystidium marinum* in oyster tissue. This culture technique enlarges Dermo hypnospores so that they may be easily visible under a microscope. Using this method, I prepared the Thio medium by

Figure 5. Red line shows measurement with calipers taken from hinge of oyster shell to beak.

Figure 6. Oyster with a sharp beak.

Figure 7. Oyster with plump meat and sharp beak.

adding 20 gm NaCl to 1 L of deionized (DI) water. I then added 29.0 g of thioglycollate to the NaCl-DI water solution and heated it on a low temperature hot plate, mixing it with a glass stirring rod by hand until all solids were dissolved. I dispensed 10 ml of this mixture with a pipette into 40 (25 ml) screw cap culture tubes. Caps were left loose on the tops of the tubes, which were then placed into test tubes racks (40 tubes each). Tubes were then autoclaved at 15 psi for 15 minutes. After the tubes cooled, the screw caps were tightened, each tube was labeled with date and time of Thio medium creation, and the tubes were then stored in the dark at room temperature until needed. Excess Thio, approximately 60 ml, was kept in a beaker and refrigerated for up to 30 days, to use if needed for additional tubes.

Preparation of Antibiotics

Later, 9 ml of deionized water was added to a 5-million-unit vial of Stock Nystatin (Sigma N6261) and shook by hand. The reconstituted mixture was allotted equally (2.5 ml) into each of 4 vials. These were labeled with the date and Nystatin Stock 1, 2, 3, or 4 and frozen (up to 365 days) until needed. To prepare the Chloromycetin/Nystatin working solution, I first added 4.5 ml of DI water to a 1gm vial of Chloromycetin (Sigma C3738, Chloramphenicol Succinate Sodium Salt) and shook it by hand to re-constitute it. The Chloromycetin solution was then added to the Nystatin Stock vial along with 17.5 ml of DI water. This mix was labeled as Chloromycetin/Nystatin working solution with date prepared and then refrigerated up to 365 days until needed. This mixture of antibiotics was necessary to prevent tissue degradation.

Preparation of Lugol's Working Solution

To prepare Lugols working solution, I added 40 ml of distilled or deionized water to 10 ml of 1N Iodine Stock solution. Iodine and Lugol's working solution were kept at room temperature in a dark cabinet until needed. Lugol's working solution serves to be used as a stain for the tissue samples.

Oyster Tissue Processing

Just before oyster tissue was added, I removed the working solution of Chloromycetin/Nystatin from the refrigerator and shook it to re-suspend the mixture. I then added 0.05 ml of the Chloromycetin/Nystatin working mixture to each Thio tube and inverted the tube to mix the solutions together. From each oyster (Fig. 8), I removed a 5-mm^2 piece of anterior mantle using a scalpel and tweezers, added it to the tube of Thio-Chloromycetin/Nystatin mixture, and labeled the tube to identify the reef and number of the oyster from which tissue was taken. Tubes to which tissue was added were stored in the dark at room temperature for a week. Then a 1-mm^2 sub-sample of the tissue in the tube was placed on a slide, masticated using tweezers, and 1–2 drops of Lugols iodine solution was applied to the tissue and blended well using the tweezers (Fig. 9). Each slide was given and identification number corresponding to its oyster and then placed in a pan (Fig. 10). I then placed a cover slip on each slide and examined the tissue under magnification (4x) using a light microscope. A Dermo prevalence rating based on the Mackin Dermo Intensity Scale (Mackin 1962) as modified by Craig et al. (1989) was recorded for each slide.

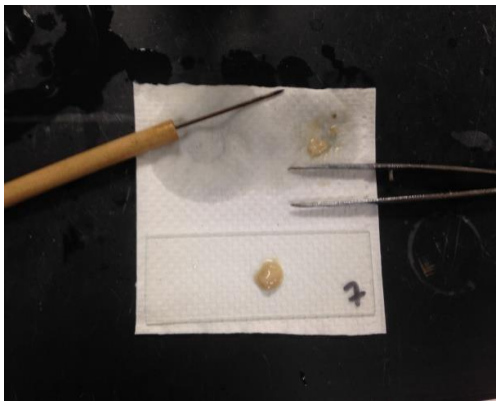

Figure 8. A 5-mm^2 piece of anterior mantle removed from an oyster and placed on a glass slide.

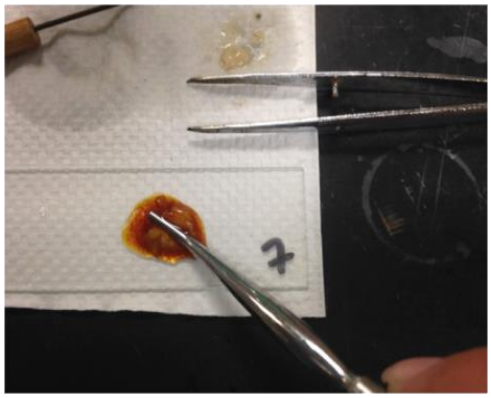

Figure 9. Tweezers used to blend Lugol's iodine solution into a 1-mm^2 sample of the oyster tissue.

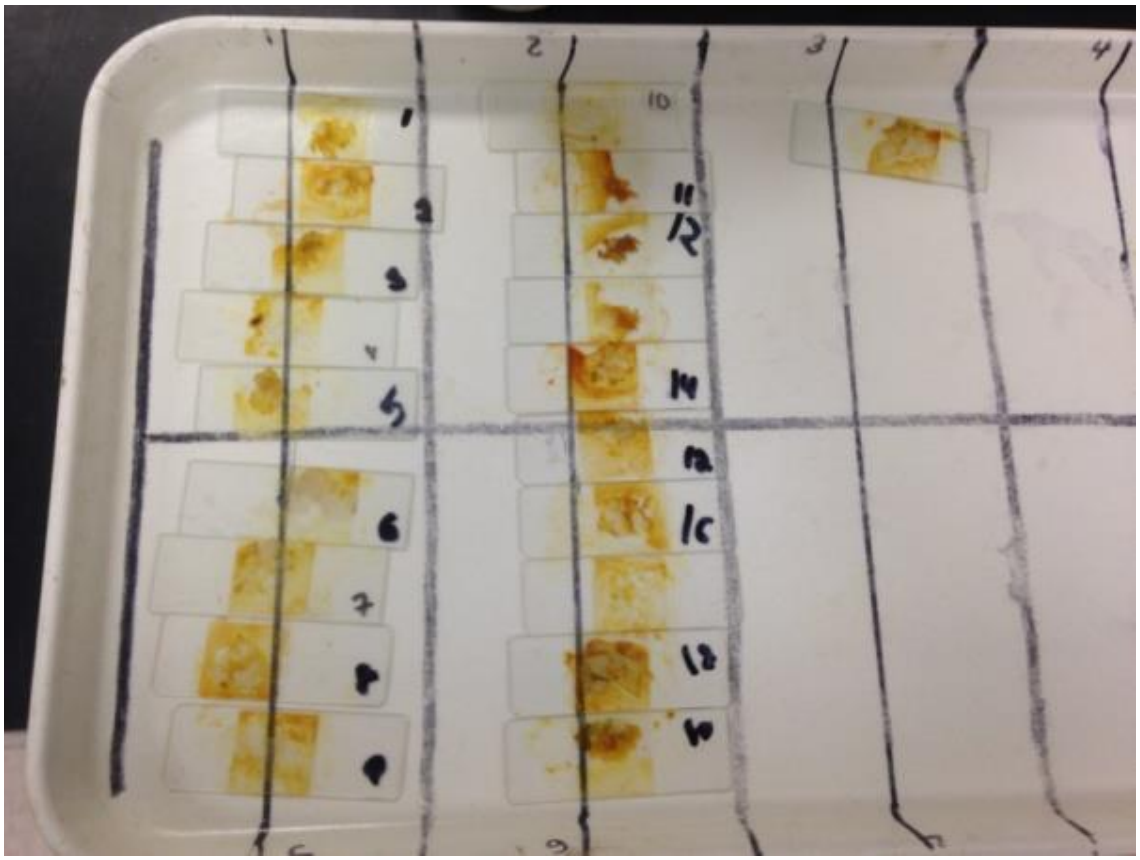

Figure 10. Oyster tissue slides in pan numbered by oyster from which they were obtained.

Mackin Dermo Intensity Scale

The Mackin Scale values (Table 1): 0 = no observable hypnospores; 1 = slight infection of tissue with hypnospores; 3 = moderate infection of tissues with hypnospores; 5 = heavily infected tissue (Mackin 1962). These prevalence ratings, along with temperature and salinity data collected at the field site were uploaded to oystersentinel.org.

Table 1. Scale of infection intensity for Dermo (*Perkinsus marinus)* (adapted from Mackin [1962] by Craig et al. [1989]).

Letter designation	Infection intensity	Numerical value	Description
N	Negative	0.00	No hypnospores present
VL	Very light	0.33	1–10 hypnospores
L-		0.67	11–74 hypnospores
L	Light	1.00	75–125 hypnospores
L+		1.33	>125 hypnospores but much less than 25% of tissue is hypnospores
LM-		1.67	<25% of tissue is hypnospores
LM	Light/moderate	2.00	25% of tissue is hypnospores
LM+		2.33	>25% but much less than 50% of tissue is hypnospores
M-		2.67	>25%, but <50% of tissue is hypnospores
M	Moderate	3.00	50% of tissue is hypnospores
M+		3.33	>50%, but much less than 75% of tissue is hypnospores
MH-		3.67	>50%, but <75% of tissue is hypnospores
MH	Moderately heavy	4.00	75% of tissue is hypnospores
MH+		4.33	>75%, but much less than 100% of tissue is hypnospores
H-	Heavy	4.67	>75% of tissue is hypnospores, but some oyster tissue is still visible
H		5.00	Tissue is 100% hypnospores

STATISTICAL ANALYSES

I used a best subsets regression analysis (MiniTab 17.0; State College, Pennsylvania, USA) to determine which individual or combination of the water-quality variables (fresh-water flow, water temperature, salinity, and turbidity) best accounted for the variation in the Mackin Dermo Intensity Scale values I obtained for my four study reefs. I also assumed fresh-water flow may have had an effect on the other 3 water variables. To illustrate these relationships, I used the scatterplot feature of "Graph" in MiniTab with a regression line.

Because there was a potential delayed effect of fresh-water flow affecting values for the Mackin Dermo Intensity Scale measurements I obtained, I used data on fresh water flow (Trinity River gage readings at Romayor, Texas located 82.5 km north of Galveston Bay) for 2 months prior to my collections. The Romayor, Texas gage was the closest gage located on the Trinity River to Galveston Bay. I then used these fresh-water flow values as a variable in my best subset regression analyses. For example, fresh-water flow in meters for the month of September 2014 was regressed with the mean Mackin Dermo Intensity Scale measurements that I recorded in November 2014.

RESULTS

Oyster Collection

April Fool Reef

During each sampling period, 20 or more oysters were dredged from the reef. Therefore, 20 of the largest oysters were kept for analysis. Oysters were generally market-sized (76 mm) or above and clumped together with barnacles found on the outside of their shells.

Fishers Reef

During each sample period at least 10 oysters were dredged from the reef. During the first two sampling trips, oysters were pulled from a mud and silt bottom, and were large and solitary. During the November 2014 collection, a commercial oyster boat was seen harvesting from the reef. During the last four sampling trips, live oysters were collected easily (only one to three passes with the dredge). The last two sampling trips brought upwards of 30 oysters in the dredge, but all the oysters were dead (Fig. 11). High mortality at this site can possibly be attributed to large fresh-water inflows starting in May 2015 (Fig. 12).

Confederate Reef

Twenty oysters were collected by hand while wading. There were numerous shore birds observed at this reef, as well as sport fish such as trout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus*).

Figure 11. A dead oyster found at Fishers Reef during July 2015 collection trip.

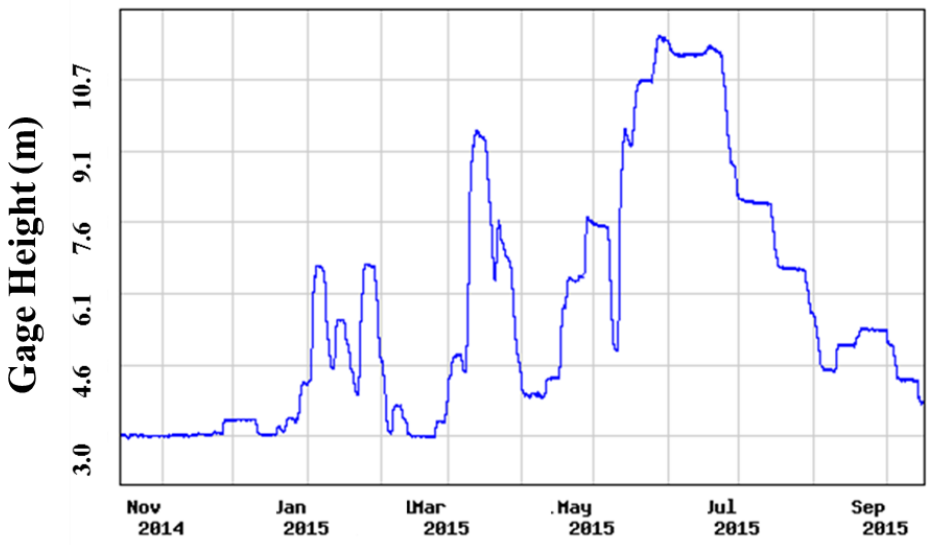

Figure 12. Height (m) of the Trinity River gage at Romayor, Texas from November 2014 through September 2015.

Frenchy's Reef

This reef was sampled a total of four times from November 2014 to May 2015. Oyster boats (Fig. 13) were observed dredging oysters at the site during November 2014 on the first sampling trip. During subsequent sampling trips, it became increasingly hard to find oysters. During May 2015, dredging yielded only six oysters, and these were attached to a piece of debris. Several dredge pulls resulted in the bringing up of debris such as shingles, glass, and plastic, and spat sized oysters. Because of the low yield of live dredged oysters, oysters from this area of Galveston Bay were substituted with

Figure 13. Commercial oyster boats at Frenchy's Reef, November 2014.

oysters dredged at the alternative site, Hannah's Reef for the remaining sample dates (July 2015 and September 2015).

Hannah's Reef

This reef was sampled once each in July 2015 and September 2015. It is situated between two private oyster leases that were identified by white PVC pipes and black flags. Twenty oysters were relatively easy to harvest (one to two pulls with the dredge). Several recreational fishing boats were observed during each sampling trip.

Dermo Prevalence

During the first sample trip in November 2014, oysters were collected and analyzed for Dermo from the original four sites in Galveston Bay. April Fool Reef exhibited an average Dermo prevalence of 0.55 on the Mackin Scale (Table 2; Fig. 14). This means there was an average of between 1–74 hypnospores in the cultured tissue sub-sample. Oysters collected at Confederate Reef had an average Dermo prevalence of 0.85 on the Mackin Scale which was an average of 11–125 hypnospores in the tissue samples collected. Fishers Reef showed an average Dermo prevalence of 0.35 on the Mackin Scale for an average between 1–74 hypnospores in the tissues sampled. Frenchy's Reef averaged 0–10 hypnospores for a Dermo prevalence of 0.05 on the Mackin Scale.

During the second sample trip in January 2015, oysters were collected and analyzed from the same four sites in Galveston Bay (Table 2). April Fool Reef had an average Dermo prevalence of 0.75 on the Mackin Scale with an average of between 11–

Table 2. Mean intensity of Dermo in oyster collected from November 2014 through September 2015 at five reefs (April Fool, Confederate, Fishers, Frenchy's, and Hannah's) in Galveston Bay, Texas. N/A refers to sample trip where reef substitution was necessary.

Date	April Fool	Confederate	Fishers	Frenchy's	Hannah's
November 2014	0.55	0.85	0.35	0.05	N/A
January 2015	0.75	0.30	0.27	0.20	N/A
March 2015	0.80	0.95	0.20	0.00	N/A
May 2015	0.60	0.00	0.43	0.45	N/A
July 2015	0.21	0.81	All dead	N/A	0.21
September 2015	0.40	1.00	All dead	N/A	0.75

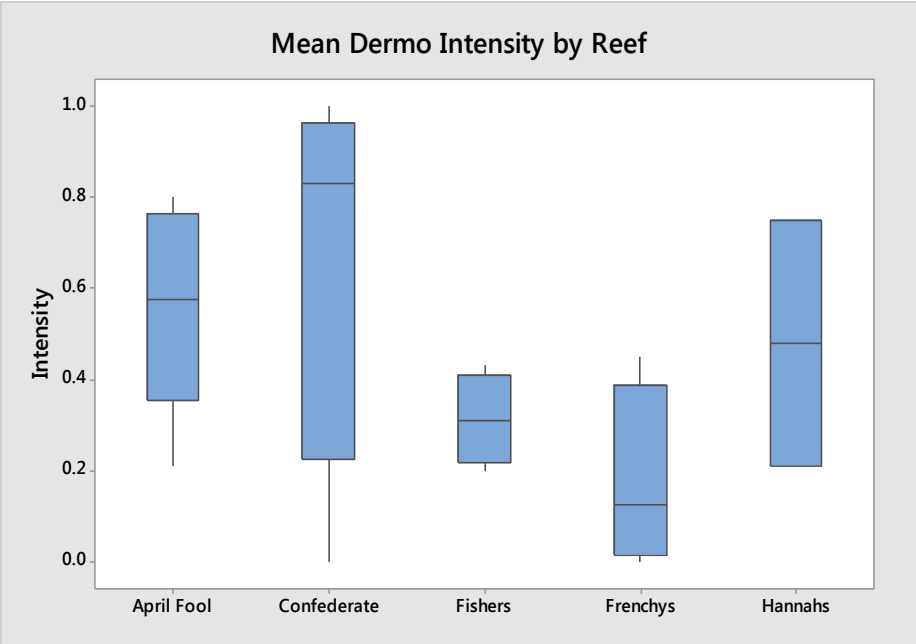

Figure 14. Mean Dermo intensity by reef.

125 hypnospores in the tissue samples collected. Confederate Reef showed an average Dermo prevalence of 0.30 on the Mackin Scale with an average of 0–10 hypnospores in

the collected tissue samples. Fishers Reef had an average Dermo prevalence of 0.27 on the Mackin Scale. The oysters collected had an average of between 0–10 hypnospores present in their tissues. Frenchy's Reef had an average Dermo prevalence of 0.20 on the Mackin Scale with an average of 0–10 hypnospores present in the tissue samples collected.

During March 2015, oysters were again collected and analyzed from the same four sites in Galveston Bay (Table 2). April Fool Reef had an average Dermo prevalence of 0.80 on the Mackin Scale with an average of between 11–125 hypnospores in the tissue samples collected. Confederate Reef had an average Dermo prevalence of 0.95 on the Mackin Scale. Oysters collected at Confederate Reef had an average of 11–125 hypnospores in the tissue samples collected. Fishers Reef had an average Dermo prevalence of 0.20 on the Mackin Scale with an average of between 0–10 hypnospores present in the collected tissue samples. Frenchy's Reef had an average Dermo prevalence of 0.0 on the Mackin Scale. There was an average of 0 hypnospores present in the tissue samples collected.

During May 2015, oysters again were collected and analyzed from four sites in Galveston Bay (Table 2). April Fool Reef showed an average Dermo prevalence of 0.60 on the Mackin Scale with an average of between 1–74 hypnospores found in the tissue samples collected. Confederate Reef had an average Dermo prevalence of 0.0 on the Mackin Scale which means there was an average of 0 hypnospores found in the tissue samples collected. Fishers Reef showed an average Dermo prevalence of 0.43 on the Mackin Scale with an average of between 1–74 hypnospores found in the collected

tissue samples. Frenchy's Reef had an average Dermo prevalence of 0.45 on the Mackin Scale, meaning there was an average of 1–74 hypnospores present in the tissue samples collected.

During the fifth sample trip in July 2015, oysters were collected and analyzed from four sites in Galveston Bay (Table 2). April Fool Reef had an average Dermo prevalence of 0.21 on the Mackin Scale meaning there was an average of between 0–10 hypnospores found in the tissue samples collected. Confederate Reef add an average Dermo prevalence of 0.81 on the Mackin Scale with an average of 11–125 hypnospores found in the tissue samples collected. All oysters collected at Fishers Reef were dead and therefore no tissue was available. Frenchy's Reef was not sampled during July 2015 because of the inability to dredge oysters from this area of Galveston Bay; therefore, oysters were dredged at Hannah's Reef for the subsequent sample dates (July 2015 and September 2015). Hannah's Reef had an average Dermo prevalence of 0.21 on the Mackin Scale. There was an average of between 0–10 hypnospores found in the tissue samples collected at Hannah's Reef.

During the sixth and final sampling trip in September 2015, oysters were collected and analyzed from four sites in Galveston Bay (Table 2). April Fool Reef showed an average Dermo prevalence of 0.40 on the Mackin Scale with an average of between 1–74 hypnospores found in the tissue samples collected. Oysters collected at Confederate Reef had an average Dermo prevalence of 1.00 on the Mackin Scale meaning there was an average of 75–125 hypnospores found in the tissue samples collected (Fig. 15). Again at Fishers Reef all oyster collected were dead and therefore

no tissue was available. Also, Frenchy's Reef was not used as a sample site during this trip and Hannah's Reef had an average Dermo prevalence of 0.75 on the Mackin Scale indicating there was an average of between 11–125 hypnospores found in the tissue samples collected.

Water Temperatures

During my sampling period, water temperatures (Table 3) at April Fool Reef ranged from 12.1^{o}C (November 2014) to 23.8^{o}C (May 2015). The average water temperature at April Fool Reef was 19.0^{o}C. Water temperatures at Confederate Reef

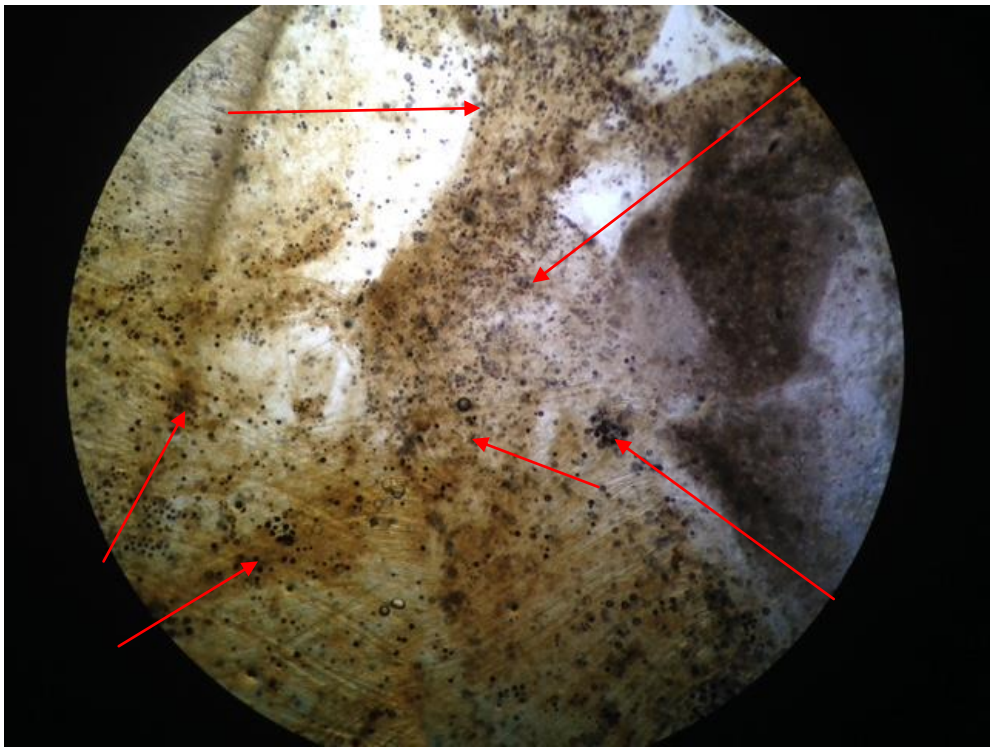

Figure 15. Dermo spores found in an oyster at Confederate Reef from the September 2015 sample.

ranged from 18.4°C (February 2015) to 32.2°C (July 2015) with an average water temperature of 24.8°C during the sampling period. At Fishers Reef, water temperatures ranged from 10.2°C (November 2014) to 23.0°C (May 2015) during the sampling period. The average water temperature at Fishers Reef was 13.6°C. At Frenchy's Reef, water temperatures ranged from 15.3°C (February 2015) to 27.3°C (June 2015). The

Table 3. Water temperature (C), salinity, and turbidity by date of oyster collection at each site.

Date	Site	Temp	Salinity	Turbidity
November 2014	Frenchy's	18.87	19.58	0.350
January 2015	Frenchy's	15.30	11.15	0.623
Mach 2015	Frenchy's	20.80	12.00	0.000
May 2015	Frenchy's	27.30	4.64	0.140
July 2015	Hannah's	30.50	3.81	0.137
September 2015	Hannah's	27.70	14.34	0.420
November 2014	Fishers	10.20	19.94	0.732
January 2015	Fishers	13.60	7.80	0.460
March 2015	Fishers	19.50	10.00	0.474
May 2015	Fishers	23.00	4.32	0.100
July 2015	Fishers	31.30	0.45	0.126
September 2015	Fishers	8.54	28.70	0.660
November 2014	Confederate	18.94	27.49	0.450
January 2015	Confederate	18.40	24.50	0.586
March 2015	Confederate	22.10	18.42	0.203
May 2015	Confederate	29.70	8.73	0.160
July 2015	Confederate	32.20	29.89	0.231
September 2015	Confederate	28.00	22.74	0.460
November 2014	April Fool	12.10	20.99	0.866
January 2015	April Fool	13.00	15.99	0.720
March 2015	April Fool	20.10	10.00	0.468
May 2015	April Fool	23.80	8.43	0.150
July 2015	April Fool	32.00	10.78	0.304
September 2015	April Fool	13.15	29.60	0.480

average water temperature at Frenchy's Reef was 20.5^{o}C. Water temperatures ranged from 27.7^{o}C (September 2015) to 30.5^{o}C (July 2015) at Hannah's Reef with an average water temperature of 29.1^{o}C for the 2 months sampled.

The overall average water temperatures were lowest at Fishers Reef (13.6^{o}C) followed by April Fool Reef at 19.0^{o}C with Confederate Reef having the highest average water temperature (24.8^{o}C). Fishers Reef was closest to the Trinity River, whereas Confederate Reef was the furthest from the Trinity River.

Water Salinity

At April Fool Reef, salinities (Table 3) ranged from 8.4 ppt (May 2015) to 20.9 ppt (November 2014). The average salinity at April Fool Reef was 15.97 ppt. Salinities at Fishers Reef ranged from 4.3 ppt (May 2015) to 19.9 ppt (November 2014). The average salinity at Fishers Reef was 11.87 ppt. Confederate Reef had salinities that ranged from 8.7 ppt (June 2015) to 29.9 ppt (July 2015). The average salinity at Confederate Reef was 21.96 ppt. Salinities at Frenchy's Reef ranged from 4.6 ppt (June 2015) to 19.2 ppt (November 2014). The average salinity at Frenchy's Reef was 11.83 ppt. Salinities ranged from 3.8 ppt (July 2015) to 14.3 ppt (September 2015) at Hannah's Reef during the sampling period. The average salinity at Hannah's Reef was 9.08 ppt.

For those reefs having salinities recorded for all 6 sampling periods, Fishers Reef had the lowest average salinity at 11.87 ppt followed by April Fool Reef at 15.97 ppt. Confederate Reef had an average salinity of 31.96 ppt. As with water flow, Fishers Reef

was closest to the Trinity River and Confederate Reef was furthest from the Trinity River.

Water Turbidity

Water turbidity (Table 3) at April Fool Reef ranged from 0.150 to 0.866 m. The average turbidity of April Fool Reef was 0.498. Turbidity at Confederate Reef ranged from 0.160 to 0.586 m. Confederate Reef had an average turbidity of 0.348. Turbidity at Fishers Reef ranged from 0.100 to 0.732 m. The average turbidity at Fishers Reef was 0.425. Turbidity at Frenchy's and Hannah's reefs ranged from 0.000 to 0.632 m. Average turbidity at Frenchy's Reef was 0.278 m and 0.279 m at Hannah's Reef.

For those reefs having water turbidity readings for all 6 sampling periods, Confederate Reef had the lowest average turbidity (0.348 m) with April Fool Reef and Fishers Reef having the highest turbidity readings (0.498 m and 0.425 m, respectively). Confederate Reef was furthest from the Trinity River where water flow probably did not increase average turbidity readings as it did at April Fool and Fishers reefs as they were closest to the Trinity River.

Relationships between Variables Collected

Best subsets regressions indicated which water variables explained differing amounts of the variability in the Mackin Dermo Intensity Scale for the reefs sampled. For April Fool Reef, the water flow gage at Romyor, Texas explained 61.8% (adjusted R-square) of the variability in the Mackin Scale (Table 4). The three water variables of temperature, turbidity, and the water flow gage explained 92.0% of the variability in the

Mackin Scale results for April Fool Reef (Fig. 16). For Confederate Reef (Fig. 17), salinity explained 20.6% of the variability in the Mackin Scale. If all water variables were included, 72.4% of the variability was explained. For Frenchy's and Hannah's reefs, water flow explained 46.9% of the variability in the Mackin Scale (Table 4). Adding water temperature to the regression only increased the explained variability to 55.7% (Fig. 18).

Because all oysters were dead for the July and September 2005 samples at Fishers Reef, I used a best subset regression using only the November 2014 and January, March, and May 2005 water variables and Mackin Scale data (Table 4). Water flow accounted for 44.5% of the variability in the Mackin Scale data (Fig. 19).

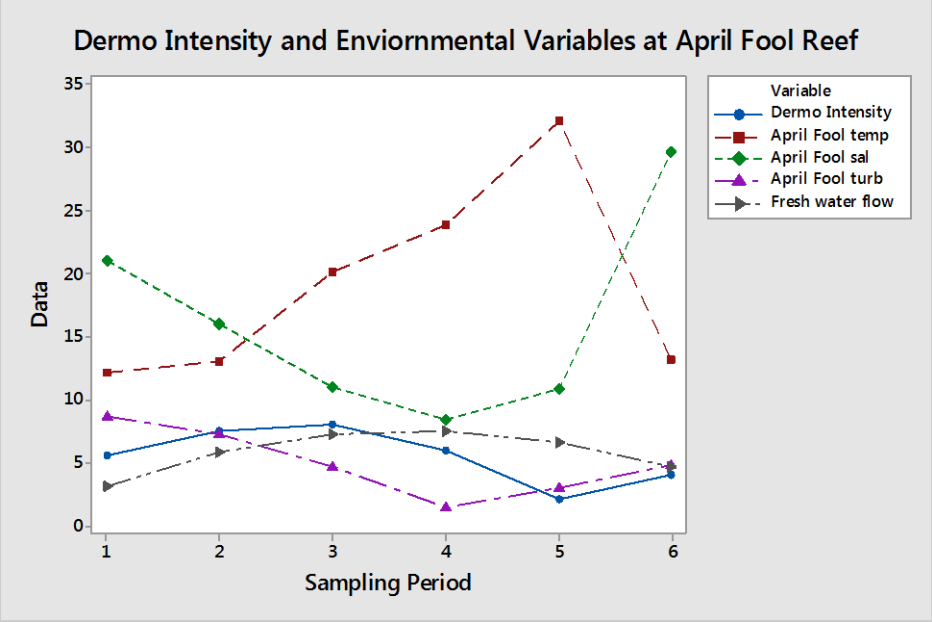

Figure 16. Graph of Dermo intensity and environmental variables at April Fool Reef.

Table 4. Best subset regression values (adjusted R-square [R-sq (adj)]) for salinity (Sal), temperature (Temp), gage height (Flow), and turbidity (Turb).

Reef	No. Varibles	R-sq (adj)	Sal	Temp	Flow	Turb
April Fool	1	61.8			X	
	1	4.5		X		
	2	83.0	X	X		
	2	65.4			X	X
	3	92.0		X	X	X
	3	88.3	X		X	X
	4	84.2	X	X	X	X
Frenchy's/Hannah's	1	46.9			X	
	1	13.2		X		
	2	55.7		X	X	
	2	53.3			X	X
	3	36.5	X	X	X	
	3	33.7		X	X	X
	4	0.0	X	X	X	X
Confederate	1	20.6	X			
	1	0.0				X
	2	3.3	X			X
	2	0.0	X		X	
	3	0.0		X	X	X
	3	0.0	X	X		X
	4	74.2	X	X	X	X
Fishers	1	44.5			X	
	1	0.0		X		
	2	95.9		X	X	

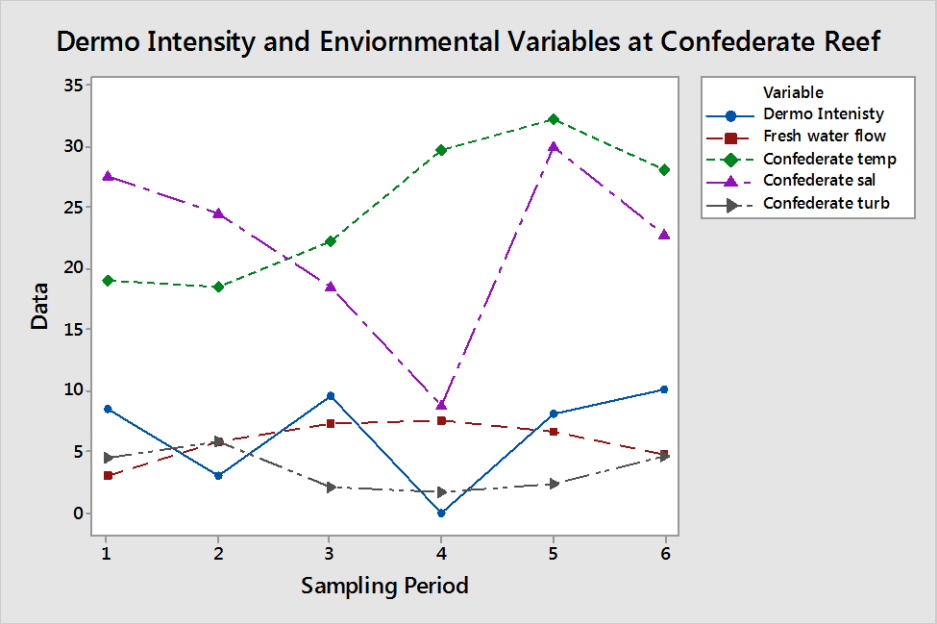

Figure 17. Graph of Dermo intensity and environmental variables at Confederate Reef.

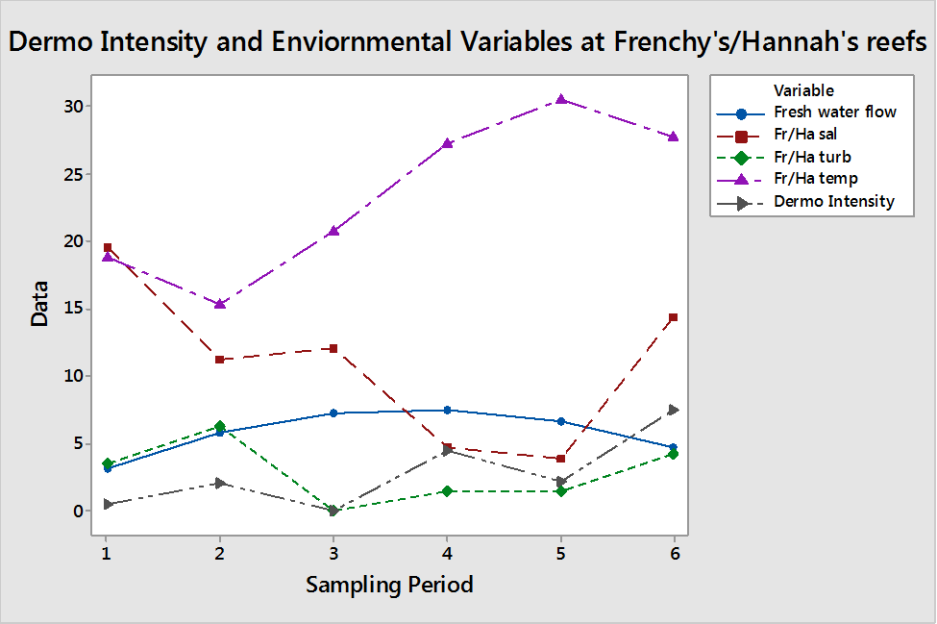

Figure 18. Graph of Dermo intensity and environmental variables at Frenchy's and Hannah's reefs.

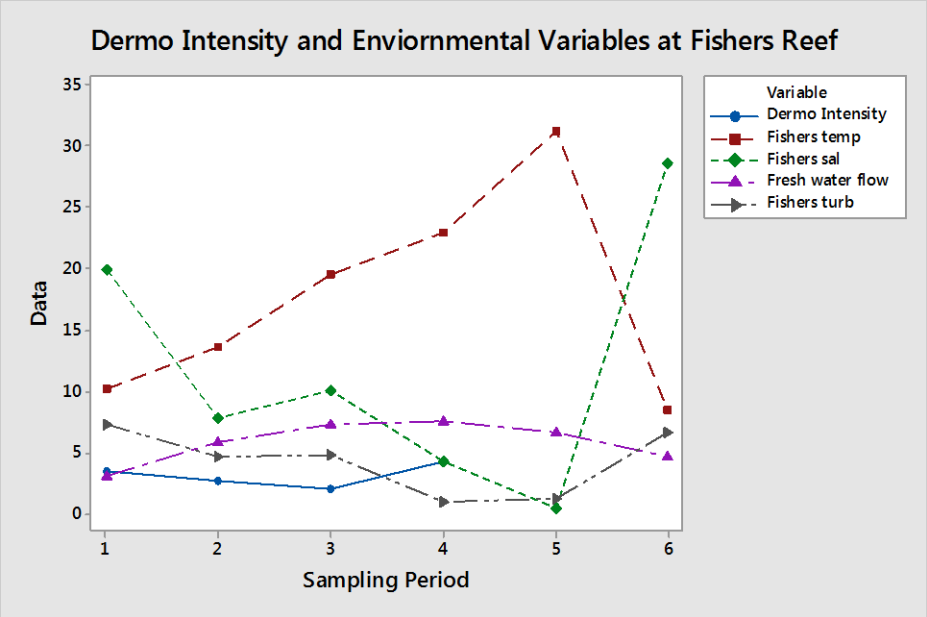

Figure 19. Graph of Dermo intensity and environmental variables at Fishers Reef

DISCUSSION

Fresh-water Inflows

The results of my study revealed that oyster reefs exposed to high fresh-water inflows had lower occurrences and intensities of Dermo infection. Reefs exposed to normal fresh-water inflows on a regular basis have lower levels of Dermo (Quigg et al 2008). However, reefs exposed to fresh-water inflows for extended periods of time (>48 hours), such as Frenchy's Reef, exhibited complete mortality. It can be concluded that fresh-water inflow exhibited the highest association and influence on disease prevalence for this reef. Water flow at April Fool Reef also attributed a majority of the variability (61.8%) in Dermo prevalence and intensities. Trinity River discharge, water temperature, and turbidity explained 92.0% of the Dermo intensities at April Fool Reef. The high percentages of variability accounted for by water flow contributed to the close proximity of both Frenchy's and April Fool Reef to the inflows from the Trinity River. For Frenchy's and Hannah's reefs, water flow explained 46.9% of the variability in the Mackin Scale. Adding temperature to the regression only increased the explained variability to 55.7%. Since both of these reefs are located in parts of the bay that are blocked from direct inflows from the Trinity River by peninsulas, it can be concluded that the influence of water flow at these two reefs were minimized. Culbertson (2008) also found that two oyster reefs she studied high to moderate amounts of dead oysters. She related this to heavy fresh-water inflows and low salinities for extended periods of time (>48 hours).

Water Salinity

The results of my study revealed that at high salinities, when combined with other variables such as extreme temperature, turbidity, and water flow, oysters sampled had a higher prevalence of Dermo. When combined, salinity accounted for higher levels of Dermo at Confederate Reef. At Confederate Reef, only salinity (20.6%) explained any of the variability in the Mackin Scale. However, when salinity, temperature, water flow, and turbidity were combined they accounted for 72.4% of the variability. Dermo is a warm water pathogen that spreads rapidly and can inundate oysters at temperatures above 25 C (Sunila 2015). Prevalence and intensity of Dermo have been found to positively correlate with salinity (Mackin 1962; Beckert et al 1972; Soniat 1985). Lower Dermo prevalence is often found in conjunction with lower salinities and high Dermo prevalence is often related to increased salinities above 25 ppt (Quigg et al. 2008). In New England, where the disease is prevalent, activity of Dermo is primarily regulated by temperature (Sunila 2015).

Water Temperature

The results of my study found as water temperature increase the prevalence of Dermo increased. For Frenchy's and Hannah's reefs, water flow explained 46.9% of the variability in the Mackin Scale scores. When water temperature was added to the regression model it increased the explained variability to 55.7%. Both of these reefs are located close to shore and protected on at least one side by Bolivar Peninsula, this could potentially decrease water flow and raise temperatures. Dermo is said to vary on a seasonal scale, with higher Dermo intensities being found in warmer months and lower

intensities found in cooler months (Quigg et al. 2008). Quigg et al. (2008) also found that at temperatures lower than 25 C there were lower Dermo intensities, and at temperatures greater than 25 C Dermo intensities were higher. In contrast, Cook et al. (1998) found that in a short term study in Delaware Bay that regression plots showed a slight increasing trend, but neither slope was statistically different from zero. Further, Ewart and Ford (1993) declared that temperature was never a limiting factor for the Gulf of Mexico.

Water Turbidity

Turbidity as a variable by itself was unimportant at all reefs in explaining the variability in the Mackin Scale intensity scores. High turbidity levels can lower amounts of dissolved oxygen and cause higher water temperatures (Behar 1997). This probably explained when water flow, temperature, and salinity were combined, 72.4% of the variability in the Mackin Scale scores was explained at Confederate Reef. There is little information known about the direct effects of turbidity on the Dermo. Oysters are said to grow best when suspended solids are in low concentrations. Sediment increase in the water column can smother larval oysters and disturb their filtration process, which can make then vulnerable to disease (Rose 1973, Cairns 1987, Chew 2002).

SUMMARY AND CONCLUSIONS

The eastern oyster (*Crassostrea virginica*) is an economically and ecologically important shellfish throughout its range, especially to the Gulf Coast of Texas. It faces a myriad of threats from abiotic and biotic sources. When oyster tissue was collected and analyzed for the presence and prevalence of Dermo disease; salinity, temperature, turbidity and fresh-water inflow, or combinations thereof, were found to affect Dermo prevalence and disease intensities.

Based on my study, the following conclusions were drawn:

1. High salinities are associated with a higher occurrence and intensity of Dermo in oyster tissue.
2. Higher amounts of fresh-water inflow were associated with Dermo disease intensity in Galveston Bay.
3. However, extreme fresh-water inflow killed oysters at Fishers Reef.
4. There is a 2-month lag time in Dermo disease reduction after heavy fresh-water inflow events in Galveston Bay.
5. The intensities and prevalence of Dermo disease in Galveston Bay increased as water temperature approached high levels (>28° C).

Based on the results of my 12 month study, I conclude that low fresh-water inflow, high salinity, and high temperatures can create conditions conducive to an increase in the occurrence and prevalence of Dermo in oysters located in Galveston Bay. I also conclude that high fresh-water inflows for a sustained period of time can cause

oyster mortality. Further, it can be concluded that low salinities and low temperatures lead to a decreased occurrence and prevalence of Dermo.

Additional research and/or longer-termed studies of the effects of salinities, temperature, fresh-water inflow, and turbidity would be beneficial to either strengthen of oppose the conclusions of my study. With further observation and testing, RFTM can be used to provide fisheries management agencies with a solid knowledge of the effects of temperature, salinity, turbidity, and fresh-water inflow on Dermo prevalence, and could be important to preventing oyster mortality, and sustaining a healthy and economically valuable population of oysters in Galveston Bay.

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